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Efficacy of simultaneous vaccination with Enterisol® Ileitis and Ingelvac® CircoFLEX™ in a Swiss breeding farm

H. Weibel[1], T. Sydler[2], E. Brugnera[1], H. Voets[3], B. Grosse Liesner[3], X. Sidler[1]

[1]Department for Farm Animals, Division Swine Medicine, University of Zurich [2]Institute of Veterinary Pathology, University of Zurich, Switzerland, [3]Boehringer Animal Health GmbH, Ingelheim, Germany

Summary

This study explores administration of two piglet vaccines as compared to the mono- and adjuvant-application. A vaccine against the Porcine Circovirus Type 2 (PCV2) cap protein subunit and a vaccine with attenuated live culture against *Lawsonia (L.) intracellularis* were applied to piglets aged 23.5 days on average. 1`405 animals were divided randomly into four groups. One piglet group was immunized with both vaccines while two other groups were immunized with a combination of one vaccine and adjuvant's of alternate vaccination protocol and vice versa. These piglet groups were also compared to a control group supplemented with both adjuvants only. During fattening, pigs, which were simultaneously immunized with Enterisol® Ileitis and Ingelvac® CircoFLEX™ vaccine, gained significantly more weight (792 g/day) when compared to piglet groups mono-vaccinated with Ingelvac® CircoFLEX™ (772 g/day) or either with Enterisol® Ileitis (774 g/day). Moreover, immunized piglet groups showed significantly higher daily weight gain when compared to adjuvants only inoculated control group (751 g/day). Additionally, during fattening the control group displayed higher mortality (6.3%) than the three vaccinated groups (Ingelvac® CircoFLEX™ 2.5%, Enterisol® Ileitis 2.3% and the combination of both vaccines 1.1%). These data imply that simultaneous immunization with PCV2- and *L. intracellularis* specific vaccines positively benefit piglet growth observed by an additive effect on growth parameters in farms harboring both pathogens. Return of investment was calculated of 2.10 on the additional Enterisol® Ileitis vaccination.

Keywords: PCV2, *L. intracellularis,* piglet vaccination, performance

Wirkung einer gleichzeitigen Verabreichung von Enterisol® Ileitis und Ingelvac® CircoFLEX™ in einem schweizerischen Zuchtbetrieb

Zusammenfassung

Die vorliegende Untersuchung verglich die zeitgleiche Applikation zweier Vakzinen an Ferkel vergleichend zu den Monoapplikationen bzw. zur Adjuvansapplikation der Kontrolltiere. Verwendet wurde an durchschnittlich 23.5 Tagen alten Ferkeln eine Subunit-Vakzine gegen das Cap Protein des porzinen Circovirus Typ 2 (Ingelvac® CircoFLEX™) und eine attenuierte Lebendvakzine gegen *Lawsonia (L.) intracellularis* (Enterisol® Ileitis). 1405 Ferkel wurden randomisiert in 4 Gruppen eingeteilt. Eine Gruppe wurde mit beiden Vakzinen immunisiert, 2 weitere Gruppen erhielten nur je eine der Vakzinen bei gleichzeitiger Applikation des Adjuvans des anderen Impfstoffes. Die Kontrollgruppe wurde nicht vakziniert, erhielt aber beide Adjuvantien. Die Tiere, die beide Vakzinen erhielten, zeigten in der Mast eine signifikant höhere Masttageszunahme (792 g/Tag) verglichen mit den Monoanwendungen (Ingelvac® CircoFLEX™: 772 g/tag bzw. Enterisol® Ileitis: 774 g/Tag). Die Ferkel mit den Monoanwendungen wiesen wiederum in der Mast signifikant erhöhte Masttageszunahmen auf im Vergleich zu der nicht geimpften und nur mit Adjuvans behandelten Kontrolltieren. Auch die Mortalität in der Mast reduzierte sich durch die Impfungen im Vergleich zu den Kontrollen (Mortalitätsraten: Kontrolltiere: 6.3%; Ingelvac® CircoFLEX™: 2.5%; Enterisol® Ileitis: 2.3%; Kombination beider Vakzinen: 1.1%). Die Daten zeigen, dass die gleichzeitige Immunisierung gegen PCV2 und *L. intracellularis* in einem Betrieb mit Infektionen mit beiden Erregern einen additiven Effekt auf die Wachstumsperfomance in der Mast bewirkt. Der Return on Investment für die zusätzliche Impfung mit Enterisol® Ileitis wurde mit 2.10 berechnet.

Schlüsselwörter: PCV2, *L. intracellularis,* Ferkelimpfung, Leistungsparamter

Introduction

Porcine proliferative enteropathy (PPE) caused by *Lawsonia (L.) intracellularis*, and porcine circovirus type 2 (PCV2) associated disease, (PCVD) (Segales et al., 2005) are an economical drag in pig production worldwide.

L. intracellularis is an obligate intracellular, gram-negative acid-resistant, bacillary bacterium (McOrist et al., 1995). PPE can be manifested acute, chronic or subclinical. In Switzerland, the chronic form of PPE is mainly observed in pigs between 6 to 20 weeks of age (McOrist and Gebhart, 1999). Clinically conspicuous signs are reduced growth, decreased feed conversion, weight inhomogeneity among animals of the same age and diarrhea. In Switzerland, a seroprevalence to L. intracellularis of 67% lies above the European average (Caspari et al., 2005). PCVD includes among other diseases postweaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS) (Segales et al., 2005). Symptoms of PMWS are wasting, profuse therapy-resistant diarrhea, dyspnea, enlarged lymph nodes, anemia and occasional icterus, as well as increase of gastric ulcers (Harding and Clark, 1997b; Allan and Ellis, 2000a). However, PMWS and chronic PPE are symptomatically hardly distinguishable. PDNS diseased animals are different since, most show skin lesions of round to irregular, red to purple macules and papules, and glomerulonephritis.

The live attenuated vaccine Enterisol® Ileitis (Boehringer Ingelheim Vetmedica GmbH, Germany) protective to PPE was approved 2005 in Switzerland. An effectivity study established vaccine potency and economic viability under Swiss breeding conditions (Caspari et al., 2009). Ingelvac® CircoFLEX™ (Boehringer Ingelheim Vetmedica GmbH, Germany) was approved for suckling piglets 2008 to PCVD prophylaxis in Switzerland., Numerous studies were conducted on the vaccine's viability globally: vaccinated animals showed better performance parameters and lower mortality rates (Fachinger et al., 2008; Kixmöller et al., 2008; Desrosier et al., 2009).

In pig production, Switzerland shows distinctive characteristics that deviate from other European countries. The farms are small with an average of 41.7 dams per breeding farm (SUISAG, 2009), and frequently by-products of the food industry will be mainly used for fattening pigs. Moreover, Switzerland is free of porcine reproductive and respiratory syndrome (PRRS), Aujeszky disease (Schwermer and Sievi, 2010), the classical and African swine fever (BVET, 2009). From 1996 to 2004, in Switzerland a coordinated effort was undertaken to eradicate and control largely all *Mycoplasma (M.) hyopneumoniae* and *Actinobacillus pleuropneumoniae (APP)* (Staerk et al., 2007) caused diseases. Since, both diseases became extremly rare.

Although Enterisol® Ileitis was efficient for mono-infected animals, in some Swiss farms with *L. intracellularis* infection the vaccine did not lead to substantial improvement. An explanation was to speculate about the presence of mixed infection with PCV2 as additionally PMWS is clinically hardly distinguishable from chronic PPE.

Therefore, we tested simultaneous administration of both vaccines Enterisol® Ileitis and Ingelvac® CircoFLEX™ in a farm with separate breeding and fattening barns with PCV2 and *L. intracellularis* infections. We compared double immunized pigs performance parameters to control groups that were immunized with one vaccine and alternate adjuvant' and vice versa, and a group with the two adjuvants only. The data indicate that the piglet group with both immunization protocols showed an additive benefit on weight performance.

Material and methods

Choice of farm

The study was conducted from May 2008 until May 2009 in a farm of sealed production systems with separate breeding and fattening barns. At the breeding farm with 58 farrowing pens and approximately 700 weaning places there was a turnover of 200 sows on yearly average. On average of 8 sows were farrowing each week. The piglets were weaned at 4 to 5 weeks of age and stayed 10 to 12 weeks at the farm.

The fattening farm contained 800 places with fully- or partly slatted floors for 7 to 14 pigs. Necropsy confirmed PCVD presence in the breeding as well as in the fattening farm according to internationally recognized criteria (Sorden, 2000; www.pcvd.org). The infection with *L. intracellularis* was detected bioScreen Ileitis antibody ELISA. Seroprevalence increased from 1.7% in week 7 to 77.6% in week 12. The farm was subjected to herd screening for PRRS viruses, salmonella, *M. hyopneumoniae, Brachyspira (B.) hyodysenteriae* and *B. psilosicoli* before begin of study and all probed animals were found negative for these pathogens.

Experimental composition

The study comprises a total of 1`565 animals in 4 test groups:

- Control group: Adjuvant for Enterisol® Ileitis and adjuvant for Ingelvac® CircoFLEX™
- Group CF: Ingelvac® CircoFLEX™ and adjuvant for Enterisol® Ileitis
- Group EI: Enterisol® Ileitis and adjuvant for Ingelvac® CircoFLEX™
- Groups EI+CF: Enterisol® Ileitis and Ingelvac® CircoFLEX™

Ingelvac® CircoFLEX™ vaccine active component is the baculovirus expressed cap protein of PCV2. It is approved for animals aged two weeks and above. Immunization protection within 2 weeks is gained by active immunization and lasts a minimum of 17 weeks. According to the manufacturer's information the Enterisol® Ileitis vaccine consists of live attenuated *L. intracellularis* (MS B3903). The freeze-dried bacteria are diluted with water and administered orally to the suckling pigs aged 3 weeks and above. This active immunization results in protection 3 weeks after vaccination.
Suckling piglets of two subsequent weeks were treated equally. The 4 groups were picked randomly within a time frame of 8 weeks. This was done thrice while test groups were always arranged randomly. All piglets pertaining to one group were taken off at the same time and not mixed with the animals of another group. The owner was only informed of group affiliation at the end of the study.

Vaccination protocol, animal identification, measurement of performance parameters and laboratory monitoring

Vaccination with Enterisol® Ileitis and Ingelvac® CircoFLEX™ or their respective adjuvants took place between day 19 and day 30. During vaccination procedures all animals were weighed, gender and heritage were noted and an electronic ISO-(International Organization for Standardization) earmark was applied. A second weighing took place in week 12 (end of weaning). Slaughter age and weight were noted directly at the slaughterhouse. Live weight at time of slaughtering was calculated based on the carcass weight divided by 79.5%.
One suckling pig per litter or at least 10 suckling pigs were randomly chosen and additionally marked. Blood samples were taken from these animals during week 3, 7, 12, 15 and 18 (EDTA-blood). At the bioScreen laboratory in Munster (Germany) the sera were tested for the presence of *L. intracellularis* antibodies via bioScreen Ileitis antibody ELISA. PCV2 virus was identified in pig blood by real-time PCR with aid of SYBR Green (Roche Diagnostics AG, Rotkreuz, Switzerland) at the Institute of

Veterinary Pathology, University of Zurich. DNA extraction was done with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics AG, Rotkreuz, Switzerland).

75% of the animals that died and 100% of animals euthanized due to their poor general condition were examined at the Institute of Veterinary Pathology, University Zurich by necropsy and histology. Mesenteric lymph node, ileum, tonsils and spleen were investigated and immunohistochemically tested for the presence of PCV2 using the F217 monoclonal antibody (McNeilly et al. 2001, Wiederkehr et al. 2009). *L. intracellularis* infection was tested by macroscopical and histological evaluation of the ileum mucosa and using histological Warthin-Starry silverstaining (W+S) to dye intracellular rods in the enterocytes of the ileal crypt cells. W+S was not applied in cases of advanced autolysis or unspectacular histology of the intestinal crypts.

Animals excluded from study

Sick animals or animals weighing less than four kilograms at weaning as well as piglets destined for breeding were vaccinated, however they were not included in the study. 66 piglets were not included due to a significantly higher average weight at time of vaccination. Other 63 piglets were also excluded from the study due to a massive outbreak of *E. coli*-diarrhoea that required peroral antibiotic treatment. Individual animals were excluded if they were not weaned with the other animals in their group, or they were moved to another administration group (n=31) later on.

Calculation of return on investment (ROI) and statistics

The economic impact of the fattening farm (week 12 to slaughtering) is expressed by the return on investment (ROI) per year, i.e. the extra profit of X Swiss francs generated per 1 Swiss franc invested. A price for slaughtered pigs of 3.60 CHF/kg (2.40 €/kg), a price of 4.40 CHF/kg (2.93 €/kg) per piglet at 30 kg and a price of 6.00 CHF (4.00 €) for both vaccines in total were taken as a basis for the calculation.

Daily weight gains were analyzed with an ANOVA without co-variables. To compare mortality rates a Chi-Square-Test was applied. SAS (Statistical Analysis System; Version 8.2, Cary, USA) software was used for statistical analysis.

Results

Group allocation

Parameters to pigs' random distribution at the beginning of the study are illustrated in table 1. No significant differences among the groups with regard to starting weight and gender division was observed.

Laboratory analysis

PCV2 DNA template concentrations and ELISA results of *L. intracellularis* antibodies from randomly selected pigs are shown in table 2. The virus titer of the groups vaccinated with Ingelvac® CircoFLEX™ remained on the base level and was one to two common logarithms lower after week 7 as compared to the unvaccinated group. There were no significant differences in *L. intracellularis* antibody values of the groups with or without the Enterisol® Ileitis vaccination. A strong increase in seroprevalence of the *L. intracellularis* antibodies between the 7th and 15th week was observed.

Mortality rates and necropsy results

During the study group sizes reduced from 1`405 animals in the beginning to 1`359 pigs in week 12 due to death (n=29) and untimely selling of animals (n=17). A further group size reduction occurred before slaughter (to n=1`246) due to deaths (n=40) and animals sold untimely (n=17). For 20 additional pigs no data could be raised due to loss of earmark; 36 animals had to be moved to another fattening stable due to space shortage after week 18.

Table 3 illustrates mortality rates during weaning and fattening period. All vaccinated group showed a significantly decreased mortality rate with the double immunized group significantly lowest mortality.

In total, 55 animals were examined at the Institute of Veterinary Pathology, University of Zurich. 28 pigs died of PCVD (Figure 1). Whereby, 22 pigs died of PMWS, 1 of PDNS and 5 of a mixed form. Intracellular rods in enterocytes of the ileo-mucosa were found in 4 animals by W+S staining. These animals also showed histological lesions typical for *L. intracellularis* infection. Three of the four W+S positive animals showed also signs of PMWS, namely, one animal from the control group and two from the group EI. The fourth animal exhibits intracellular rods pertained to administration group EI+CF and died of a right ventricle insufficiency. Of the other 27 animals examined, 9 died of intestinal torsion, 5 died due to diarrhea at the age between 37 and 56 days. In one of these pigs *E. coli* (O141:K85) was confirmed. For

the other 13 animals various diseases independently of PCV2 and *L. intracellularis* infections were diagnosed.

Daily weight gain

Table 4 illustrates daily weight gain during fattening period. Average daily weight gain (ADWG) during fattening was significantly higher in the immunized groups compared to control group. The combined vaccinated group EI+CF showed significantly higher ADWG (+ 41 g/d) than single immunized group EI (+ 23 g/d) or CF (+ 21 g/d). However, ADWG was not improved after vaccination in the weaning period. Interestingly, slight reduction of ADWG was seen even in some vaccinated groups, which was more than compensated during fattening. Hence, the economic benefit of Enterisol® Ileitis with lower mortality rate (- 1.4 %) and augmented ADWG (+ 20 g/d) on the level of an already PCV2 immunized pig population in the fattening period is illustrated by the ROI of 2.10.

Discussion

During fattening period it was obvious that the simultaneous administration of Enterisol® Ileitis and Ingelvac® CircoFLEX™ (group EI+CF) led to significantly higher average daily weight gain (ADWG) and reduced mortality rates as compared to the control and to mono vaccinated groups (groups CF and EI). Ingelvac® CircoFLEX™ vaccinated group showed significantly reduced mortality, increased ADWG and none died by PCVD. Also, Enterisol® Ileitis vaccinated group reduced significantly mortality and increased ADWG.

Side effects with the simultaneous application of Enterisol® Ileitis and Ingelvac® CircoFLEX™ were not systematically checked; however, the owner as well as the respective veterinarian did not observe any negative reactions including sudden death, systemic allergic reactions and local swelling in all vaccinated groups (more than 1000 pigs). Furthermore, the combined vaccination supported the best performance.

The impact of the Ingelvac® CircoFLEX™ vaccination was also seen in the PCV2 genome content of the blood. The virus titer of the groups vaccinated with Ingelvac® CircoFLEX™ was one to two common logarithms lower after week 7 as compared to the unvaccinated group. This led most probably to a decrease in PCV2 infection

pressure on the stock and one may even assume that unvaccinated control animals profited from the PCV2 infection pressure decrease. Similar PCV2 genome values were described in earlier studies (Cline et al., 2008; Fachinger et al., 2008).

The increase in PCV2 genome concentrations between week 7 to week 12 in animals not vaccinated with Ingelvac® CircoFLEX™, and the seroconversion against *L. intracellularis* after week 7 led to the conclusion that vaccination during week 3 was early enough for both vaccines. Considering the allocation of deaths during weaning and fattening time, the increase in PCV2 genome content between week 7 to week 12 PCV2 specific immunization prevented PCVD mortality in the fattening period.

Groups that received an immunization (CF group and EI group) showed significantly higher ADWG during fattening period compared to control group (Enterisol® Ileitis +22g/day; Ingelvac® CircoFLEX™ +21g/day). These results are in agreement with various other studies (Fachinger et al., 2008; Kixmöller et al., 2008; Caspari et al., 2009). Nevertheless, the combination of the immunization (group EI+CF) attributed to the best growth on individual pig with an average of +41g/day on top of the control group' growth during the fattening period.

Seroprevalence of *L. intracellularis* in Swiss farms is high at 67% and this fact is interpreted that *L. intracellularis* infection might often appear only in a subclinical form. In the investigated farm only few animals were diagnosed clinically suspicious for PPE, probably most animals were subclinically infected with *L. intracellularis*. This assumption is further supported by the considerable less ADWG during fattening period of the control group and EI or CF group compared with double vaccinated group (EI+CF group). Thus, this combined vaccination strategy against PCV2 and *L. intracellularis* will reduce antibiotic application and more importantly will significantly increase growth and concomitantly decrease pigs mortality. In spite of increased production costs the economic benefit of the combined vaccination outweighs reflected by ROI of 2.10 by the additional Enterisol® Ileitis.

Table 1: Administration groups with number of animals (n), starting weight, -age and gender division.

Parameters	Control group	CF	EI	EI + CF	Total
Number of animals (n)	327	376	318	384	1`405
Weight at vaccination (kg)	6.54	6.60	6.59	6.78	6.63
Age at start (day p.p.)	23.4	23.1	23.1	24.2	23.5
Female (%)	42.2	39.6	41.5	39.3	40.6
Male, castrated (%)	57.8	60.4	58.5	60.7	59.4

Table 3: Mortality rates during weaning and fattening period.

Time	Parameters	Control	CF	EI	EI+CF	Total
Total	Number of animals (n)	327	376	318	384	1`405
	Mortality rate (%)	8.3[a]	4.0[b]	5.3[ab]	2.6[b]	4.9
Weaning	Number of animals (n)	327	376	318	384	1`405
	Mortality rate (%)	2.1	1.6	3.1	1.6	2.1
Fattening	Number of animals (n)	318	367	306	368	1`359
	Mortality rate (%)	6.3[a]	2.5[b]	2.3[b]	1.1[b]	2.9

Indices a/b mark deviations between the administration groups with a significant level of $p \leq 0.05$.

352 Table 4: Daily weight gains during fattening period (week 12 to slaughtering).

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Parameters	Control group	CircoFlex	Enterisol	EI+CF
Number of animals (n)	283	312	292	359
Daily weight gains during fattening (g/day)	751[a]	772[b]	774[b]	792[c]

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355 Indices a/b/c mark deviations between the administration groups with a significance level of $p \leq 0.05$.

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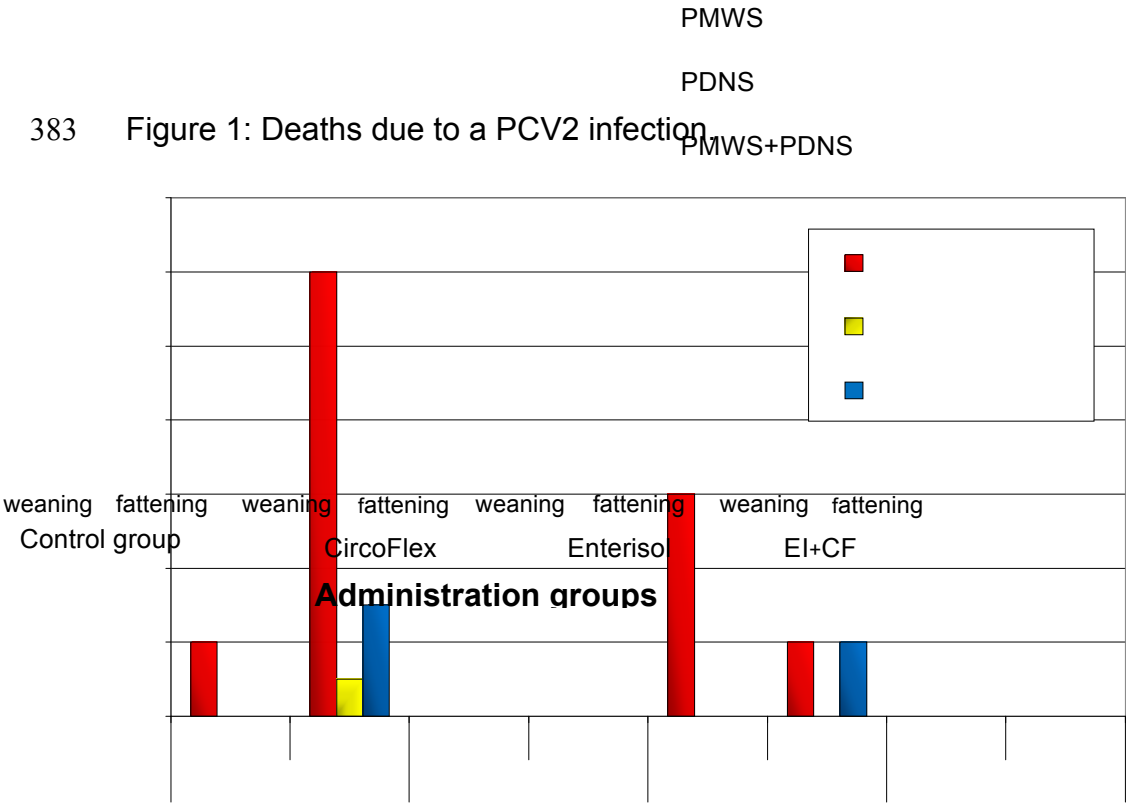
Table 2: *L. intracellularis* specific antibodies in serum and PCV2 DNA template concentrations (as log 10 of PCV2 DNA template content per ml) in pig blood, separated according to age.

week	*Lawsonia antibodies (%))* positive	negative	doubtful	*PCV2 genomes* with CircoFLEX™	without CircoFLEX™
3	4.4	91.6	4.0	4.2	4.1
7	0.9	96.3	2.7	4.4	4.5
12	76.6	14.0	9.3	4.1	5.8
15	90.9	4.3	4.8	4.3	6.1
18	84.6	6.5	9.0	4.1	6.0



383 Figure 1: Deaths due to a PCV2 infection

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